



Proteomic Analysis of BAL from Diesel Exposed Rats

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Disclaimer



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Outline



- NIOSH Rat Lavage Study
 - » SELDI-TOF at NIOSH
 - » Protein Identification at USACEHR
- Technical difficulties that have arisen
 - » SELDI-TOF data
 - » Scoring algorithm for protein identification



Diesel Exposure



- Rat study with Diesel Exhaust Particles (DEP)
 - » Intratracheal instillation of particles
 - » 0, 5, 35, or 50 mg DEP / kg body weight
 - » Harvest bronchoalveolar lavage(BAL)
 - 1, 7, 30 days post exposure
- Protein Profile by SELDI-TOF at NIOSH
- Protein Identification at USACEHR
 - » SDS-PAGE and ion trap
 - » LC-MS on QTOF



SELDI-TOF



- Surface Enhance Laser Desorption Ionization

- » Uses laser to ionize samples dried onto a plate

- Primarily singly charged ions

- » Has specificity on plate

- we used WCX Chips
(Weak Cation Exchange)

- Time of Flight

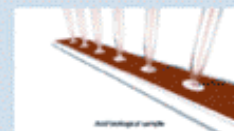
- » Allows analysis of full length proteins

- » Top down proteomics

Ciphergen's SELDI ProteinChip® Process

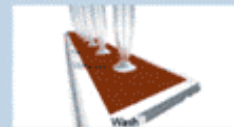
1. Apply Crude Sample

Proteins bind to chemical or biological "docking sites" on the ProteinChip Array surface through an affinity interaction.



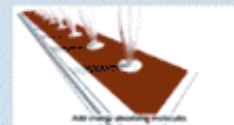
2. Wash ProteinChip Array

Proteins that bind non-specifically and buffer contaminants are washed away, eliminating sample "noise."



3. Add Energy Absorbing Molecules

After sample processing, the ProteinChip Array is dried and EAM is applied to each spot to facilitate desorption and ionization by SELDI-TOF-MS.



4. Analyze in a ProteinChip Reader

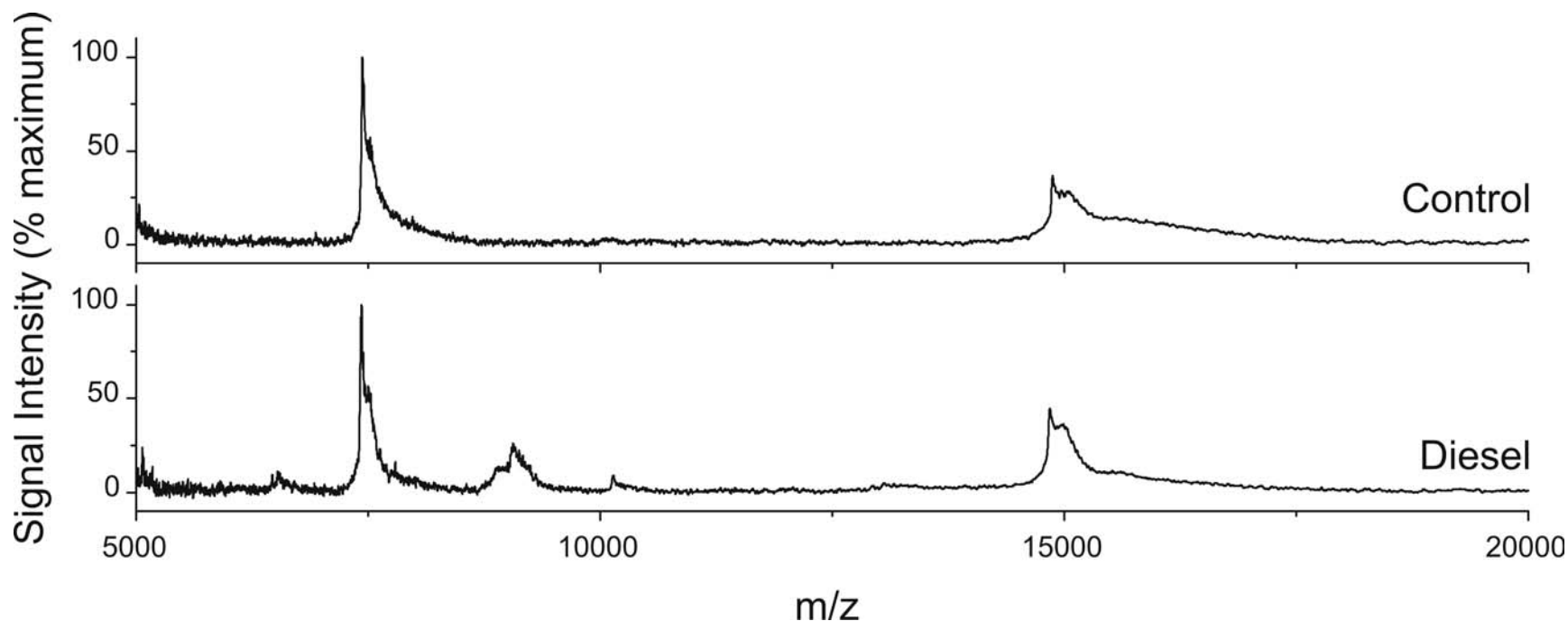
The proteins that are retained on the ProteinChip Array are detected in the ProteinChip Reader.





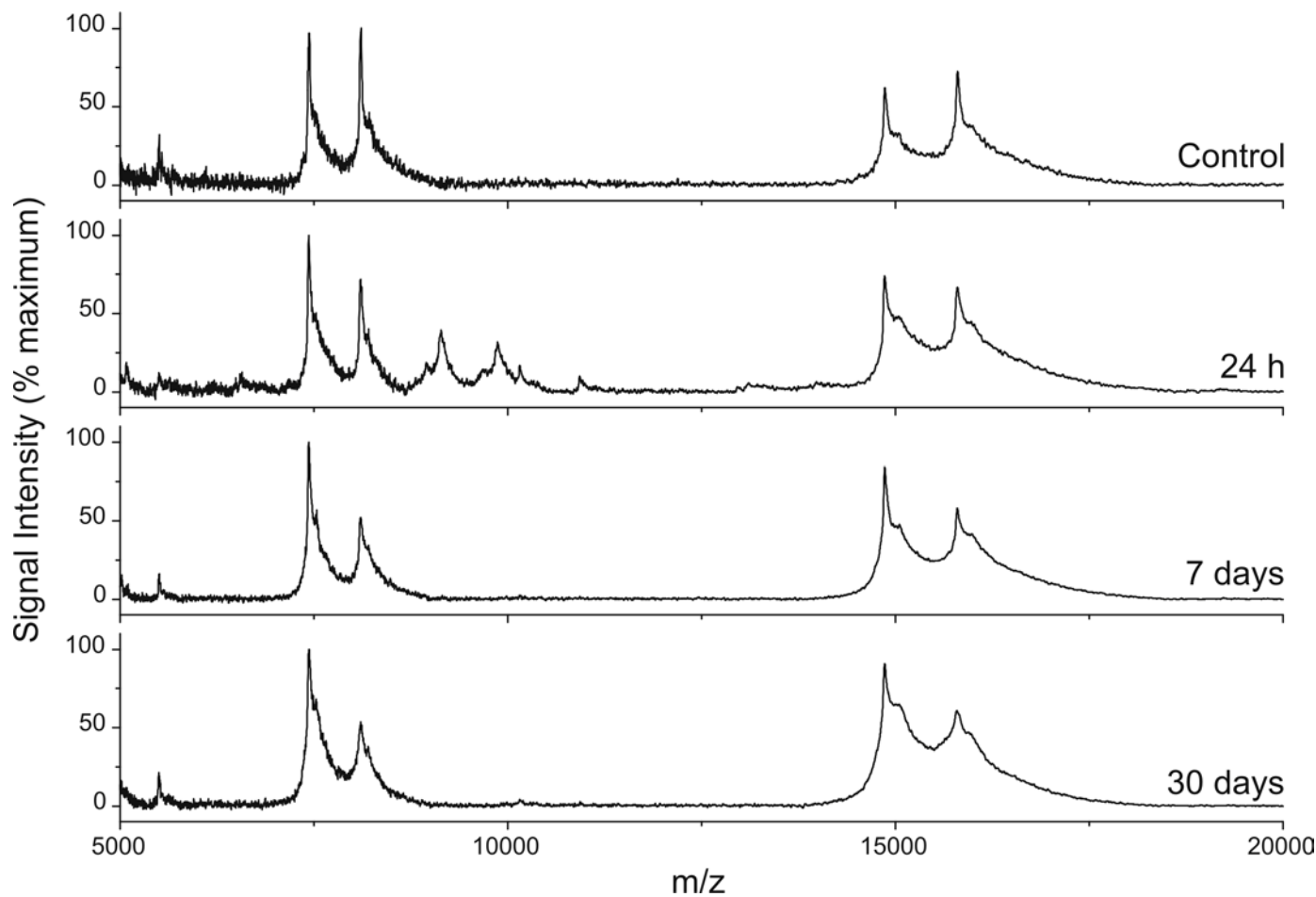
SELDI Results

- 2 peaks present in all doses at 24 hrs
 - » 9,100 and 10,100 m/z
- Absent at other time points



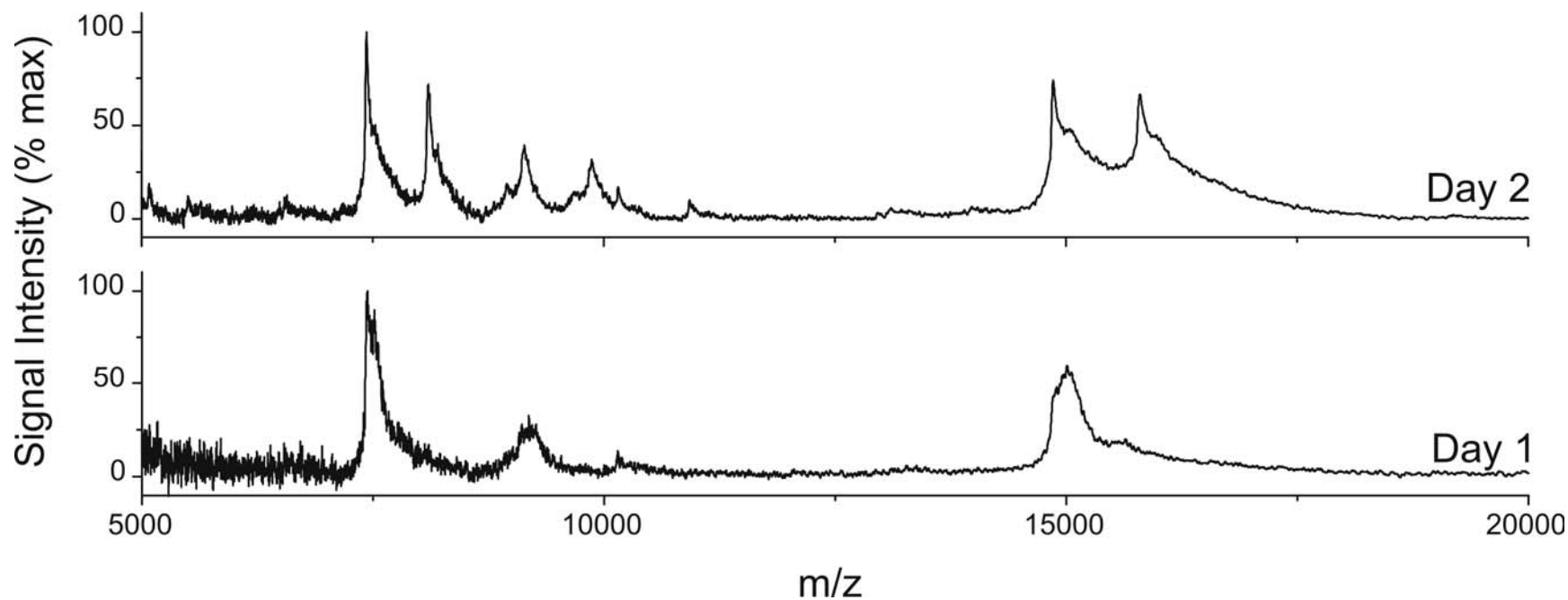


SELDI of Time Course



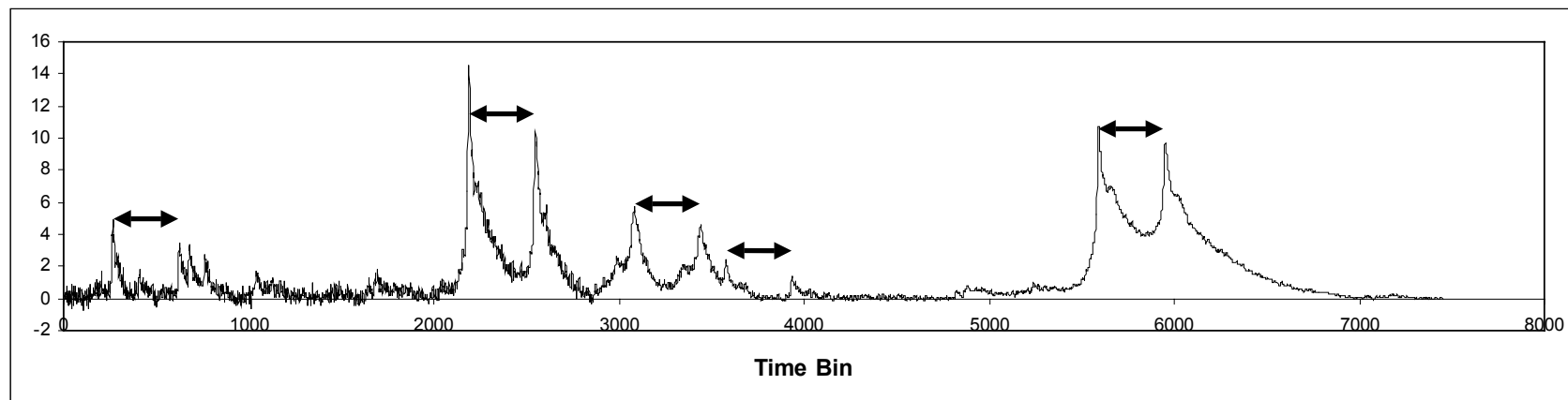
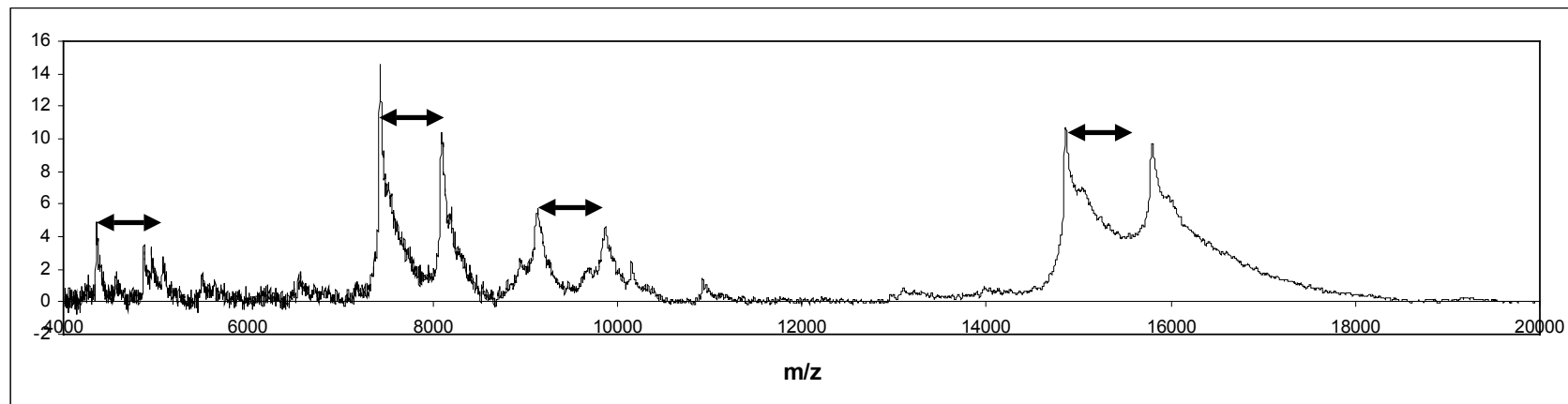


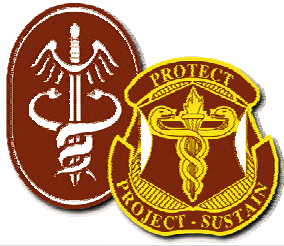
SELDI Doublets





Doublet Spacing





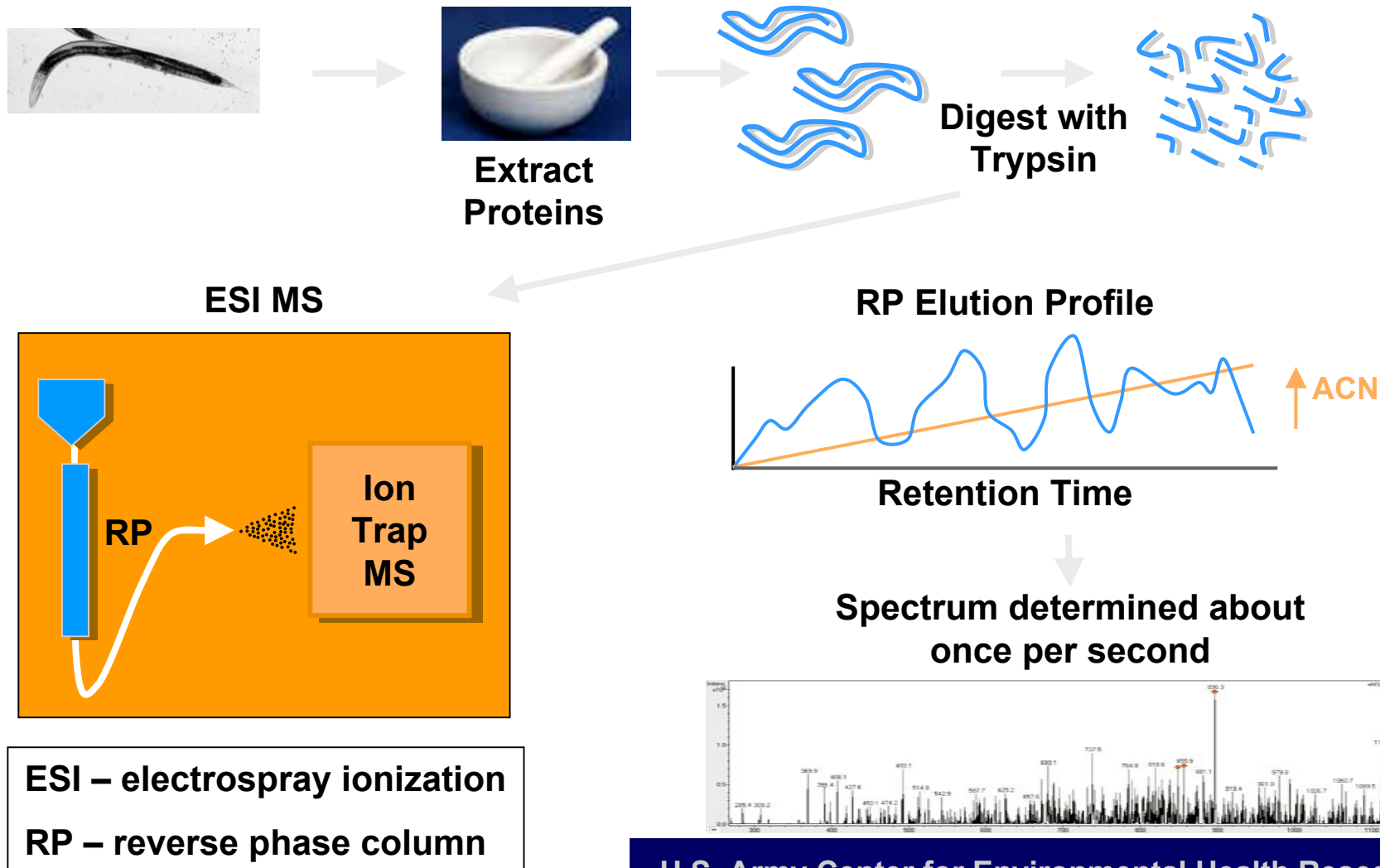
USACEHR's Task



- Identify protein at 9,100 and 10,100 m/z
 - » Extract to mimic WCX chip
 - » SDS-PAGE
 - » Analysis with ion trap
- Global analysis of BAL fluid
 - » QTOF



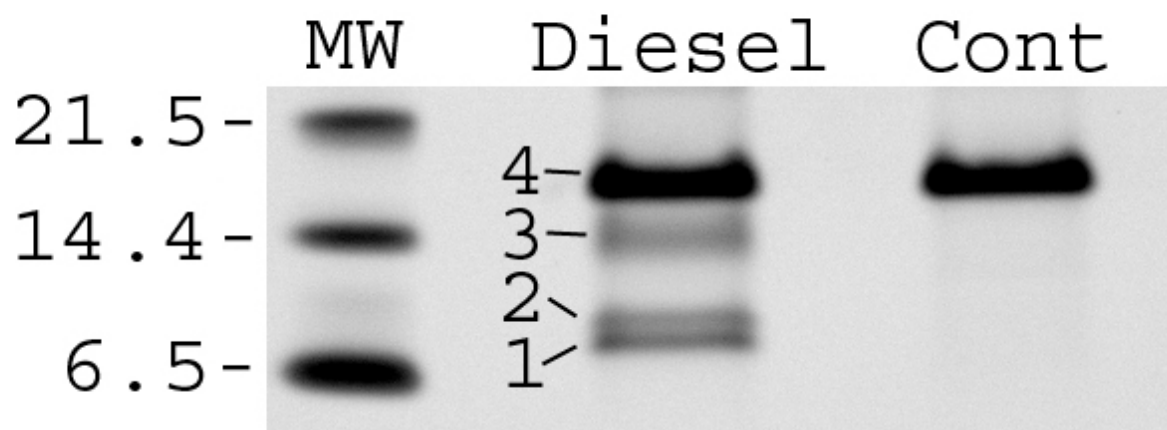
LC-MS work flow





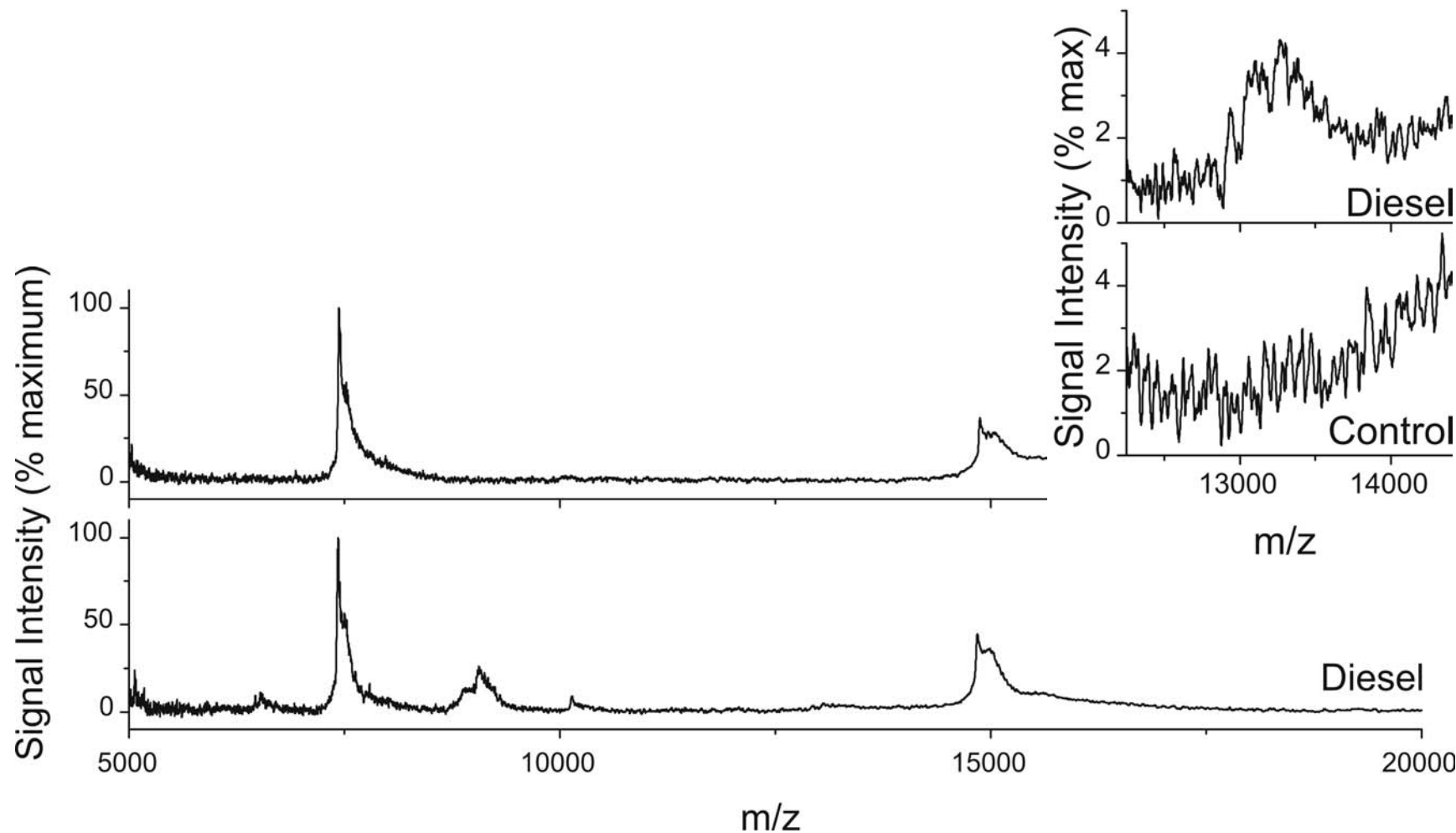
SDS-PAGE

- Extract with CM resin
- Run SDS-PAGE
- Excise protein bands, digest and elute
- Analyze on ion trap
- Search database for sequence identities



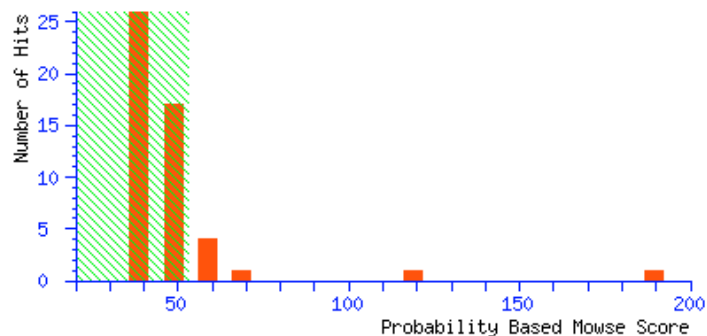


Extra Peak





Search Results



Peptide Summary Report

1. [40254796](#) Mass: 17175 Score: 189 Queries matched: 10

lysozyme [Rattus norvegicus]

☐ Check to include this hit in error tolerant search or archive report

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
716	823.52	822.51	822.42	0.09	0	(26)	8.1	4	R.DLSGYIR.N
717	823.53	822.52	822.42	0.10	0	(22)	21	9	R.DLSGYIR.N
<input checked="" type="checkbox"/> 718	412.27	822.53	822.42	0.10	0	43	0.16	1	R.DLSGYIR.N
<input checked="" type="checkbox"/> 719	412.29	822.57	822.42	0.14	0	(34)	1.4	1	R.DLSGYIR.N
788	458.69	915.37	915.47	-0.11	0	(28)	5.1	2	R.AWVAWQR.H
<input checked="" type="checkbox"/> 791	458.72	915.43	915.47	-0.05	0	(31)	2.8	1	R.AWVAWQR.H
792	458.79	915.57	915.47	0.09	0	(31)	2.9	2	R.AWVAWQR.H
793	458.83	915.65	915.47	0.17	0	33	1.9	2	R.AWVAWQR.H
<input checked="" type="checkbox"/> 608	730.46	2188.36	2187.98	0.38	0	69	0.0031	1	R.NYNPGDQSTDYGFQINSR.Y
<input checked="" type="checkbox"/> 757	872.65	2614.93	2614.23	0.70	0	48	0.44	1	K.NACGIPCSALLQDDITQAIQCAK.R + IMID_light (K)



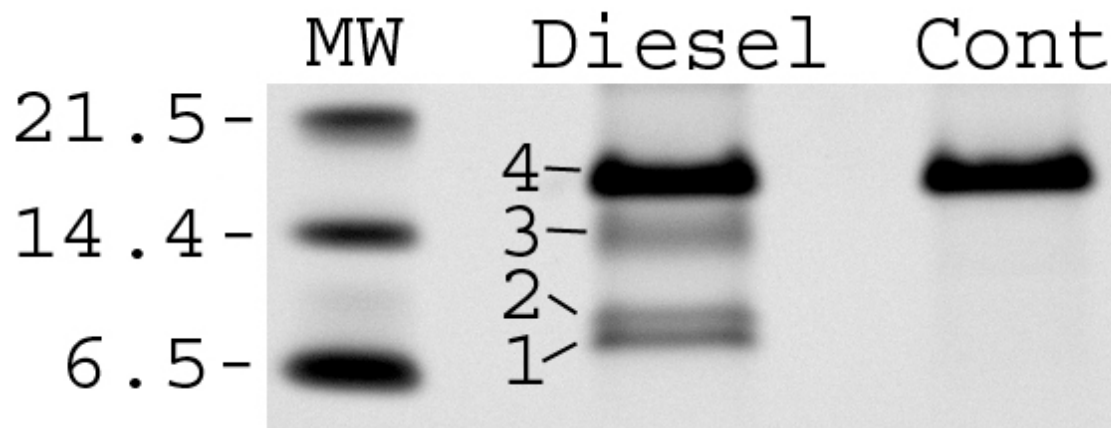
Protein Identifications



Gel Band	High Score ^a	Number Of Spectra	Labels Applied	Sequence	Mods Detected	Protein
1	6	1	IMID	ILLQGTPVAQMAEDAVDGERLK		C3
1	56	2	IMID	LITQGESCCLK	IMID	C3a
1	34	1		AFMDCCNYITK	ox-Met	C3a
1	34	1		LITQGESCCLK		C3a
1	87	6		MVTTECPQFVQNK	ox-Met	cal A
1	66	1		MVTTECPQFVQNK		cal A
2	3	1	IMID	FGLEKR		C3
2	11	1	IMID	ARLITQGESCCLK	IMID	C3a
2	79	6	IMID	LITQGESCCLK	IMID	C3a
2	64	3		AFMDCCNYITK	ox-Met	C3a
2	59	6		LITQGESCCLK		C3a
2	64	1		MVTTECPQFVQNK	ox-Met	cal A



SDS-PAGE



Band	Protein ID
4	Lysozyme
3	-
2	Calgranulin A
1	Anaphylatoxin C3a



Additional Analysis



- Analysis of proteins by LC-MS
 - » Use QTOF
 - » Extracted and total BAL
 - » Verify protein identification
 - » Quantitative and Qualitative comparison



Protein ID for Extracted BAL

- Samples extracted using weak cation exchange resin
- Analyzed on QTOF
 - » Use Water's Proten Expression Method
 - No precursor selection
- Scoring Criteria
 - » Present in all 3 technical replicates
 - » = 0.99 confidence score
 - » > 2 peptide per protein

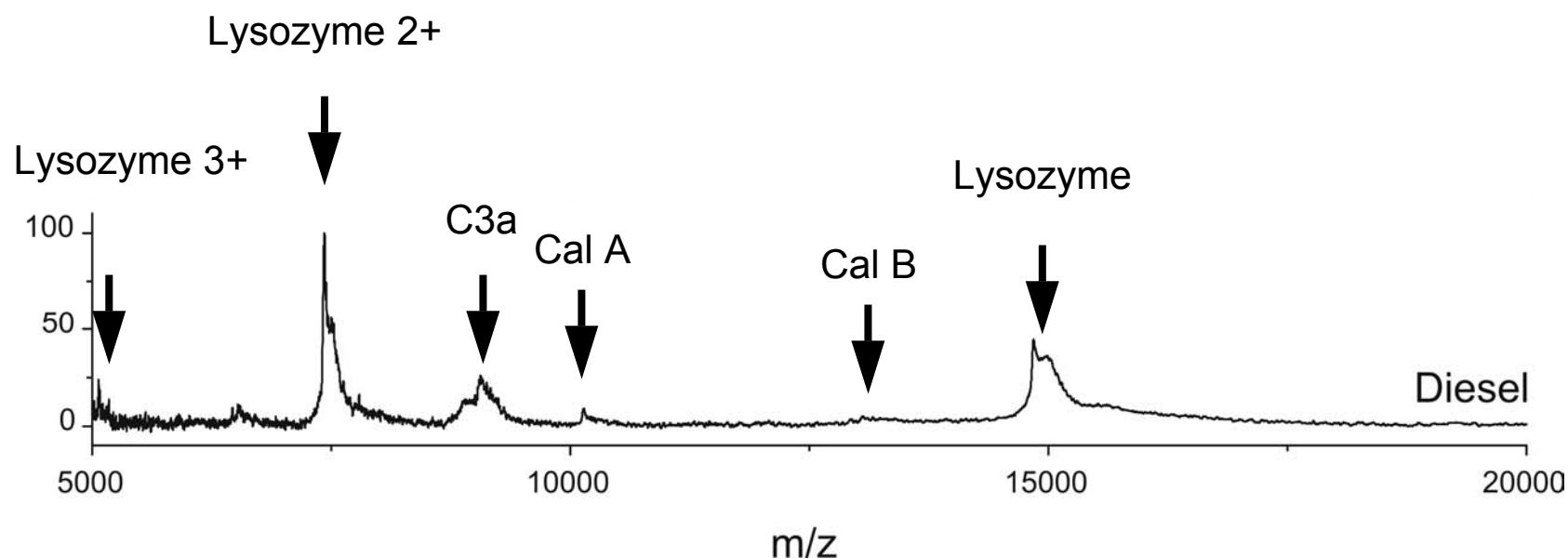


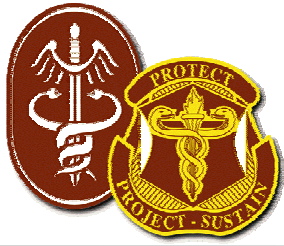
Extracted BAL Results

Replicate	Condition	Peak m/z	Confidence	Score	Unique Peptides	Protein
1	Exposed	9,100	1	58.9	3	calgranulin A
2	Exposed		1	49.0	4	calgranulin A
3	Exposed		1	51.3	4	calgranulin A
1	Exposed	10,100	1	51.8	25	complement C3
1	Exposed			60.5	33	XP_579384
2	Exposed		1	39.8	26	complement C3
2	Exposed			38.7	26	XP_579384
3	Exposed		1	107.5	29	XP_579384
1	Exposed	13,200	1	43.4	3	calgranulin B
2	Exposed		1	53.0	7	calgranulin B
3	Exposed		1	30.8	4	calgranulin B
1	Exposed	5,000 7,500 & 15,000	1	107.0	7	lysozyme
2	Exposed		1	61.3	6	lysozyme
3	Exposed		1	120.8	7	lysozyme
1	Unexposed		1	56.4	5	lysozyme
2	Unexposed		1	58.0	5	lysozyme
3	Unexposed		1	92.9	8	lysozyme



SELDI Peak Identifications





Biological Meaning

- Anaphylatoxin C3a
 - » Cleavage product of Complement C3
 - » Complement C3 and XP_579384 - 98% identity
 - » Mediates inflammation response
 - » Part of alternate complement pathway
- Calprotectin
 - » Heterodimer of Calgranulin A and Calgranulin B
 - » Very abundant in polymorphonuclear leukocytes
 - » Currently used as a biomarker of inflammation



Protein ID for Total BAL



- Fractionate peptides prior to LC-MS analysis
 - » 8 fractions from HPLC
- Present in all 3 technical replicates
- ≥ 0.99 confidence score
- > 2 peptide identified per protein
- False positive rates too high
 - » Include random sequences in database



Additional Scoring Criteria

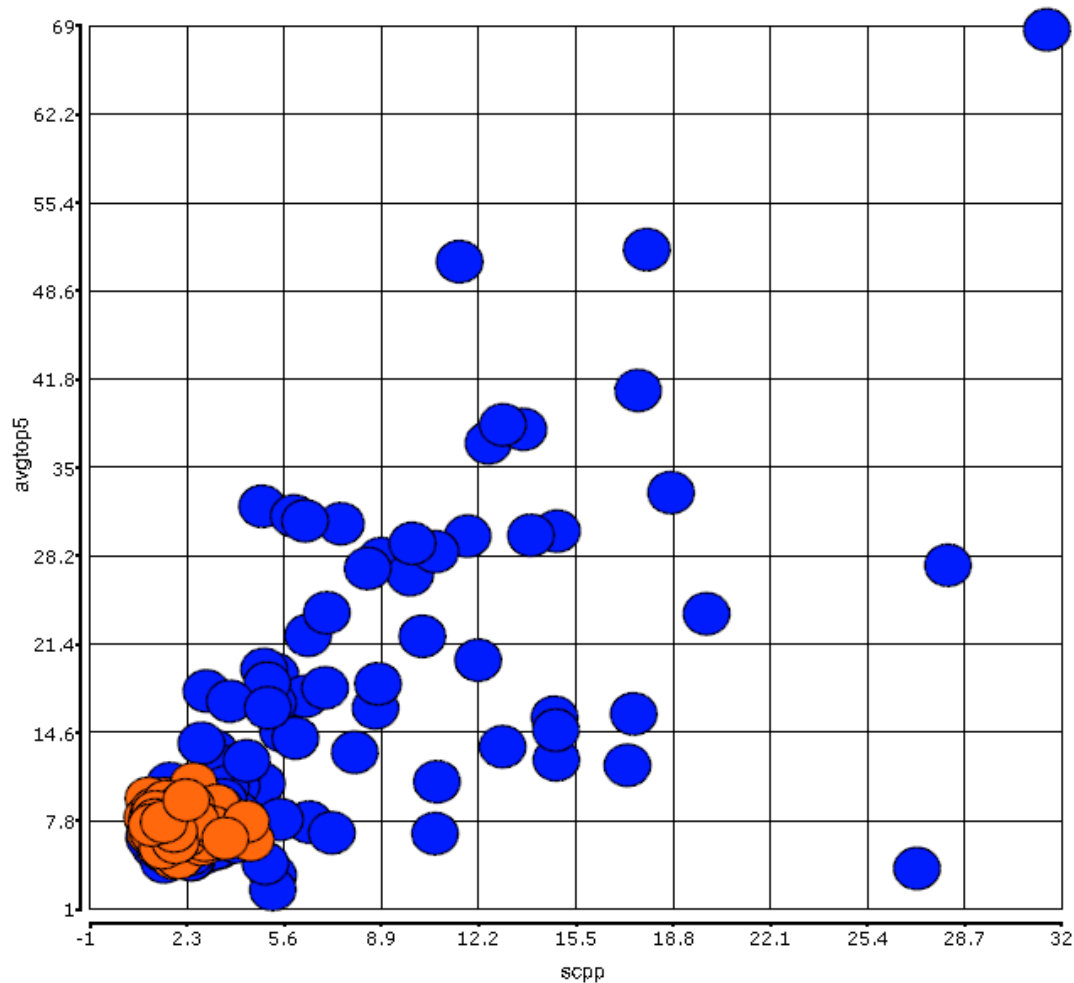


Score / Peptide > 2.7

Top 5 Average > 10

False Positives

- ~ 5 % for single run
- BDL with replicate filter





Additional Findings

- LC-MS protein identifications
 - » Identified 65 proteins
 - » Checked against unfiltered search results to find lower quality identifications
 - 20 in Diesel Exposure only
 - 4 in Control only
 - Relevant ??



Biological Meaning

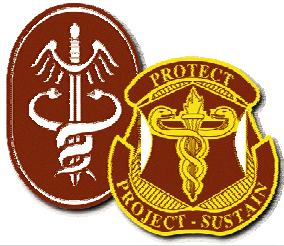


- Many proteins are plasma derived and found in both diesel and control
 - » More abundant in diesel
 - » Extravasation after exposure
 - Sham might be causing some also
- Proteins found only in diesel exposure (20)
 - » Lung Damage and Inflammation Proteins



Conclusion

- SELDI was able to identify changes
- Top down approach allowed identification of cleavage product, anaphylatoxin
- Global approach produced more candidates
- Inflammation response and extravasation dominate protein sample
- List of potential protein biomarkers
 - » Need to verify in serum or plasma



Acknowledgements



- USACEHR
 - » Bill Dennis
 - » Paul Knechtges
 - » Dave Jackson

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 - » Val Vallyathan
 - » Vince Castranova